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European Patent Office
Office européen des brevets

Publication number:

0 204 179
B1

EUROPEAN PATENT SPECIFICATION

Date of publication of the patent specification:
08.11.89

Application number: 86106456.6

Date of filing: 13.05.86

Int. Cl.: **C12P 1/06, C12P 21/06,**
C07K 15/14
// (C12P1/06, C12R1:045)

Method for selectively increasing the ratio of single major components of telcoplanin A2 complex.

Priority: 21.05.85 GB 8512795

Date of publication of application:
10.12.86 Bulletin 86/50

Publication of the grant of the patent:
08.11.89 Bulletin 89/45

Designated Contracting States:
AT BE CH DE FR GB IT LI LU NL SE

References cited:
EP-A-0 137 506
GB-A-2 121 401
US-A-4 239 751

THE JOURNAL OF ANTIBIOTICS, vol. 37,
no. 6, 1984 (Tokyo), A. BORGHI et al. "Telchomycins,
new antibiotics from actinoplanes telchomyceticus
Nov. Sp. IV, Separation and characterization of the
components of telchomycin (Telcoplanin)", pp. 615-620

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EP O 204 179 B1

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Description

Teicoplanin (formerly named teichomycin) is a glycopeptide antibiotic produced by cultivating *Actinoplanes teichomyceticus* nov. sp. ATCC 31121. This antibiotic is active mainly against infections by gram-positive bacteria.

According to the procedure described in U.S. 4,239,751, teicoplanin is isolated from the fermentation broths of the producing strain as a complex containing three factors named A₁, A₂ and A₃. Factor A₂ is present in preponderant amount in the antibiotic complex recovered from the fermentation of the above strain and is the most important for its biological effects. Factor A₁ and factor A₃ are present only in minor amount.

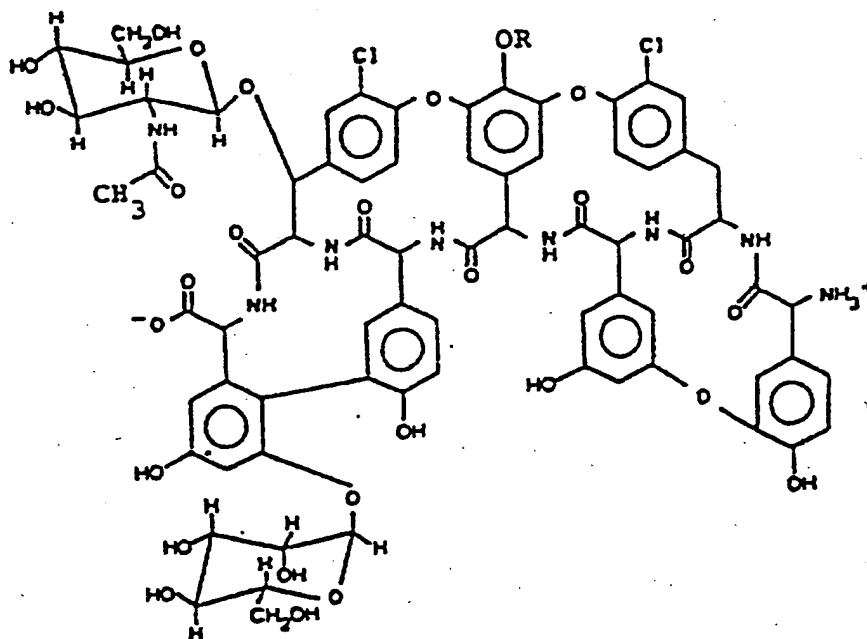
According to U.S. 4,239,751, teicoplanin A₂ (T-A2) is isolated from the other factors of teicoplanin complex by column chromatography on Sephadex^R LH-20, which is a hydroxypropyl derivative of a cross-linked polydextran gel with an exclusion limit at about molecular weight 4,000.

From large scale preparation and purification operations (examples of these operations are given in European Patent Application Publication No. 0122969) it is usually obtained a teichomycin product essentially consisting of teicoplanin A₂ accompanied by a small quantity of teicoplanin A₃. This product is suitable for practical use in therapeutical applications. See: *Drugs of the Future*: Vol. 9, No. 6, 1984, pages 429-430 edited by J.R. Prous Publishers, Barcelona, Spain.

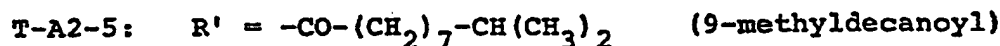
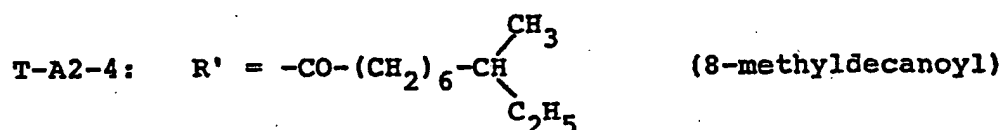
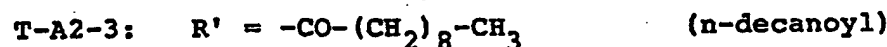
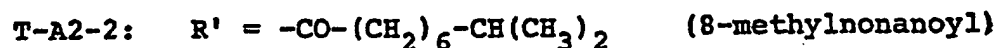
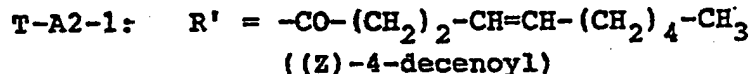
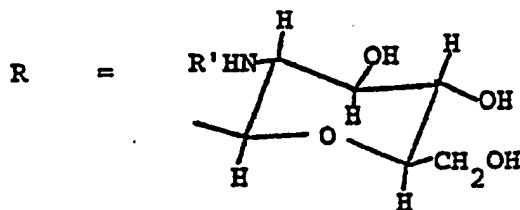
A paper published by A. Borghi, C. Coronelli et al. in *Journal of Antibiotics* Vol. 37, No. 6 pp. 615-620, June 1984, teaches that teicoplanin factor A₂ (T-A2) is, in turn, a mixture of five closely related major components of very similar polarity.

These components, designated as T-A2-1, T-A2-2, T-A2-3, T-A2-4 and T-A2-5, were isolated by using, in a first step, reverse phase partition chromatography at normal pressure on a silanized silica gel column. The purification of components T-A2-3, T-A2-4 and T-A2-5 required a further step with the application of semi-preparative HPLC on a Whatman Partisil^R ODS M-9 column eluted with a 0.2% aqueous ammonium formate-acetonitrile mixture (76:24). All said components have been chemically and biologically characterized. See also British patent application publication No. 2,121,401.

Structural elucidations reported by J.C.J. Barna, D.M. Williams et al. in *J. Am. Chem. Soc.* 1984, 106, 4895-4902, show that the teicoplanin A₂ major components may be represented by the following structural formula:



where



In vitro and *in vivo* tests reported in the above mentioned British patent application publication No. 2,121,401, show that each of the T-A2-2, T-A2-3, T-A2-4 and T-A2-5 components is more active than the telcoplanin A₂ complex as a whole.

It is therefore apparent that a method for selectively enhancing the production of each of the major components of telcoplanin A₂ is a primary objective in telcoplanin industrial fermentation. The significant technical advantage it may offer concerns both the purpose of isolating the single T-A2 major components in a pure form and the possibility of obtaining a T-A2-complex enriched with the more active components. Moreover, the possibility of modulating the ratio of the single T-A2 major components in the T-A2 complex in the large scale industrial fermentation, offers a useful tool to maintain constant the composition of the fermentation product which must adhere to standard specifications. In other words, when for any reason (e.g., a modification of the industrial culture medium to employ less expensive materials), the percent composition of the single components tends to depart from that of the standard, the possibility of selectively increasing each of the T-A2 major components offers a useful tool to correct such a defect.

The object of this invention is to provide a method for selectively increasing the ratio of the single T-A2 major components in the T-A2 complex. More particularly, the object of this invention is a process for obtaining telcoplanin A₂ selectively enriched in any of its major components T-A2-1, T-A2-2, T-A2-3, T-A2-4 and T-A2-5 which consists in adding to the culture medium of *Actinoplanes telchomyceticus* nov. sp. ATCC 31121 or a mutant thereof which produces T-A2 complex through the same metabolic pathway, a selectively effective amount of an appropriate precursor of the characteristic acyl group linked to a glucosamine moiety of T-A2 (see the above meanings for R'), hereinafter: "appropriate precursor of the respective acyl group of the glucosamine moiety of T-A2".

The process of this invention is characterized in that:

a) the appropriate precursor for increasing the ratio of T-A2-1 in T-A2 complex is selected from linoleic acid, its salts with bases which are non-toxic to the microorganism and its esters with mono- and polyhydroxy lower alkanols

b) the appropriate precursor for increasing the ratio of T-A2-2 in T-A2 complex is selected from valine, its salts with acids and bases which are non-toxic to the microorganism, alpha-keto-isovaleric acid,

its salts with bases which are non-toxic to the microorganism, its esters with mono- and poly-hydroxy lower alkanols, isobutyric acid, its salts with bases which are non-toxic to the microorganism, its esters with mono- and poly-hydroxy lower alkanols, isobutanol and its esters with acids which are non-toxic to the microorganism

5 c) the appropriate precursor for increasing the ratio of T-A2-3 in T-A2 complex is selected from oleic acid, its salts with bases which are non-toxic to the microorganism, and its esters with mono- and poly-hydroxy lower alkanols

d) the appropriate precursor for increasing the ratio of T-A2-4 in T-A2 complex is selected from isoleucine, its salts with acids and bases which are non-toxic to the microorganism, alpha-keto-beta-methylvaleric acid, its salts with bases which are non-toxic to the microorganism, its esters with mono- and poly-hydroxy lower alkanols, 2-methylbutyric acid, its salts with bases which are non-toxic to the microorganism, its esters with mono- and poly-hydroxy lower alkanols, 2-methylbutanol and its esters with acids which are non-toxic to the microorganism

10 e) the appropriate precursor for increasing the ratio of T-A2-5 in T-A2 complex is selected from leucine, its salts with acids and bases which are non-toxic to the microorganism, isovaleric acid, its salts with bases which are non-toxic to the microorganism, its esters with mono- and poly-hydroxy lower alkanols, alpha-keto-isocaproic acid, its salts with bases which are non-toxic to the microorganism, its esters with mono- and poly-hydroxy lower alkanols, isoamyl alcohol and its esters with acids which are non-toxic to the microorganism.

15 Salts with bases which are non-toxic to the microorganism are salts wherein the type and concentration of the given cation is such that it does not impair the growth of the microorganism culture or the production of the desired antibiotic substance to a considerable extent. Examples of said cations are sodium, potassium, ammonium and the like.

20 Esters with mono- and poly-hydroxy lower alkanols are (C₁-C₆)alkanols with 1, 2, 3, 4, 5 or 6 hydroxy functions per molecule.

When (C₄-C₆)alkanols are used, they must be different from those which act as precursors for other T-A2 major components (e.g. isobutanol, isoamyl alcohol, and 2-methylbutanol) unless concomitant increase of one or more of said components is desired.

30 Preferred examples of poly-hydroxy alkanols are glycerol and propylene glycol.

When the lower alkanol is present in different enantiomeric and epimeric forms, in the present description and claims, both each single form separately and the mixture of the single forms in any proportion are intended.

35 Esters which are non-toxic to the microorganism are (C₂-C₂₂)alkanoyl esters wherein the type and concentration of the alkanoyl moiety in the fermentation medium is such that it does not impair the growth of the microorganism culture or the production of the desired antibiotic substance to a considerable extent. In general, straight chain (C₂-C₄)alkanols are preferred.

40 The method of this invention involves cultivating the above mentioned strain in an aqueous nutrient culture medium containing an assimilable source of carbon, an assimilable source of nitrogen and inorganic salts under the usual conditions described in the prior-art for the production of telcoplanin, with the improvement that a selectively effective amount of an appropriate precursor is added to the fermentation medium before inoculation of the strain or during the fermentation process to selectively increase the production of one or more of the telcoplanin A₂ components T-A2-1, T-A2-2, T-A2-3, T-A2-4 and T-A2-5.

45 The expression "a mutant thereof which produces T-A2 complex through the same metabolic pathway" refers to those natural or artificial mutants of *Actinoplanes teichomyceticus* ATCC 31121 (parent strain) which produce the T-A2 complex by using essentially the same enzymatic systems as the parent strain to provide the R' fatty acyl moiety of the T-A2 complex.

50 In this specification and in the claims the expression "selectively effective amount" means a quantity of selective precursor which, when added to the culture medium, yields a concentration of selective precursor sufficient to produce the selective increase of a specific component of T-A2 complex without causing toxic effects to the microorganism.

55 The nutrient fermentation media suitable for the fermentation of T-A2 producing strain which can be used in the embodiment of this invention usually contain: a suitable carbon source which, for instance, may be selected from sugars (e.g. glucose, sucrose, maltose), polysaccharides (e.g. starch, dextrane) and polyalcohols (e.g. glycerol, propylene glycol); a suitable nitrogen source which, for instance, may be selected from ammonium salts, asparagine, peanut meal, soybean meal, meat extract, tryptone, peptone, yeast hydrolyzate, yeast extract and corn steep liquor; acid mineral salts such as sodium chloride, calcium carbonate, magnesium sulfate.

60 The fermentation is carried out for a time varying from 50 to 200 hours under aerobic conditions at a temperature between 25°C and 35°C, preferably between 27°C and 33°C. The addition of the selectively effective amount of appropriate precursors can be made to the fermentative media before inoculation of the producing strain, however, it is preferably made 24 to 48 hours after the fermentation is started. The addition may be made in one or several portions or in a continuous way.

65 According to a typical experiment embodying this invention, the *Actinoplanes teichomyceticus* strain

maintained on oat-meal agar slants is inoculated into a flask containing 100 ml of vegetative medium. After 36 hours, samples of the culture (5 milliliters) are used to inoculate a series of fermentation flasks containing 100 ml of fermentative medium. After 24 to 48 hours of fermentation the selectively effective amount of precursor is added as appropriate. If concomitant increase of two or more major components of T-A2 complex is desired, two or more precursors can be added to the same fermentation flask. The fermentation is continued for additional 60 to 150 hours, the medium is centrifuged off and samples of the broth are analyzed for T-A2 major components concentration by high performance liquid chromatography (HPLC).

The addition of the precursor is generally made in a way that may not alter the pre-determined pH value of the fermentation medium. Thus, for instance, when free acid precursors are added directly to the medium, the pH value is maintained under control by buffering the medium or by immediate neutralization with bases which are non-toxic to the microorganism.

When the precursor to be added is an amino acid, it may be supplied to the fermentation medium as an aqueous solution of its salts with acids or bases which are not toxic to the producing microorganism, e.g. hydrochlorides and sodium salts. Both racemic mixtures and optically active isomers can be used as precursors.

However, at least in some instances, the addition of the L-form gives higher yields than the corresponding D-form.

A preferred embodiment of the process of this invention is represented therefore by the use of the L-amino acid precursor for enhancing the concentration of T-A2-2 (valine, a salt or an ester thereof), T-A2-4 (L-isoleucine, a salt or an ester thereof) and/or T-A2-5 (L-leucine, a salt or an ester thereof) of teicoplanin A₂ complex. According to this preferred embodiment, it is also possible to increase the percentage of T-A2-2, T-A2-4 or T-A2-5 in the fermentation product up to 90-95% of the complex.

With lower alkanolic acid precursors (e.g. isobutyric acid, 2-methylbutyric acid, isovaleric acid, alpha-keto-isovaleric acid, alpha-keto-beta-methylvaleric acid, and alpha-keto-isocaproic acid) the addition may be made through an aqueous solution of their salts with non-toxic bases; ammonium and sodium salts are usually preferred.

When salts of unsaturated fatty acids, such as linoleic acid and oleic acid, are used as the appropriate precursor, sodium and ammonium salts are generally preferred. However, any salt with a base which is not toxic to the producing strain may be employed.

When esters of the above lower alkanolic acids and unsaturated fatty acids with mono-hydroxy lower alkanols are employed as precursors, said esters are usually derived from methanol, ethanol and propanol, although esters with C₄-C₆ alkanols may also be employed. In this case, the C₄-C₆ alkanol must be different from those which may act as precursors for other T-A2 major components (e.g. isobutanol, isoamyl alcohol, and 2-methylbutanol) unless concomitant increase of one or more of said components is desired.

Preferred esters of the above lower alkanolic acids and unsaturated fatty acids with poly-hydroxy lower alkanols are the esters with ethylene glycol and glycerol, e.g. triisobutylin, tri-oleine and tri-linoleine.

The addition of unsaturated fatty acids can be carried out also by using natural raw materials containing said acids as such or their glycerides. For instance, commercial soybean oil usually contains about 20 to about 35 percent of oleic acid and about 50 to about 60 percent of linoleic acid as triglycerides; lard contains about 40 to about 55 percent of oleic acid; cotton seed oil contains about 20 to about 45 percent of oleic acid and about 30 to about 55 percent of linoleic acid; sun flower seed oil contains about 15 to about 25 percent of oleic acid and about 65 to about 75 percent of linoleic acid.

Alkanol precursors such as isobutanol, isoamyl alcohol and 2-methylbutanol are usually added as such to the fermentation medium. However, they can be supplied also as esters of acids which are non-toxic to the microorganism. These acids must be different from those which may act as precursors for other T-A2 major components (e.g. isobutyric acid, isovaleric acid, 2-methylbutyric acid, linoleic acid, etc.) unless concomitant increase of one or more of said components is desired. Usually, esters with linear lower alkanolic acids such as acetic, propionic and butyric acid are preferred.

The "selectively effective amount" to be added to the fermentation medium according to this invention depends on the type of precursor. Usually, with the esters of the lower alkanolic acids (isobutyric acid, 2-methylbutyric acid, isovaleric acid) and the esters of unsaturated fatty acids (linoleic acid, oleic acid), amounts to yield a concentration into the fermentation medium ranging between 0.5 g/l and 15 g/l are employed with the range between 1 g/l and 5 g/l being preferred. With the lower alkanols (isobutanol, 2-methylbutanol, isoamyl alcohol) or their esters with acids which are non-toxic to the microorganism, amounts to yield a concentration ranging between 0.5 g/l and 5 g/l are usually employed, with the range between 1 g/l and 2 g/l being preferred.

With the amino acids (e.g. valine, leucine, isoleucine) and the keto-acids (alpha-keto-isovaleric acid, alpha-keto-beta-methylvaleric acid, alpha-keto-isocaproic acid) or their salts with acids and bases the "selectively effective amount" added to the fermentation medium usually ranges between 0.5 g/l and 5 g/l, with the range between 1 g/l and 3 g/l being preferred.

In the case where the lower alkanolic acids (e.g. isobutyric acid, 2-methylbutyric acid, isovaleric acid), the unsaturated fatty acids (e.g. linoleic acid, oleic acid) or their salts are directly added to the fermenta-

tion medium, the "selectively effective amount" usually ranges between 0.1 g/l and 2.5 g/l, with the range between 0.3 g/l and 1.5 g/l being preferred.

High r concentrations are still effective in promoting the selective increases of the T-A2 major components but the overall yield of T-A2 complex is depressed because of toxic effects on the microorganism.

5 The following examples describe in detail some specific embodiments of this invention.

Example 1

General Procedure

10 One oat meal agar slant of *Actinoplanes teichomyceticus* nov. sp. ATCC 31121 was inoculated into a 500 ml flask containing 100 ml of the following vegetative medium:

Glucose 10 g/l

Peptone Difco 4 g/l

15 Yeast extract 4 g/l

MgSO₄ 0.5 g/l

CaCO₃ 5 g/l

Standard oligo elements 1 ml of each of the solutions A, B and C

Water 1000 ml

20 (pH adjusted to 6.7 after sterilization)

Solution A: 10% sodium chloride (w/v)

Solution B: 10% calcium chloride (w/v)

Solution C: H₃BO₃: 50 mg; CuSO₄: 4 mg; KI: 10 mg; FeCl₃: 20 mg; MgSO₄: 40 mg; FeSO₄: 40 mg; (NH₄)₂MoO₄: 20 mg; in 100 ml of distilled water.

25 After 36 hours of growth on a rotary shaker, five milliliters of the culture were used to inoculate the test flasks and standard flasks containing each 100 ml of fermentation medium having the following composition:

Yeast lysate 5 g/l

Asparagine 1.5 g/l

30 Glucose 20 g/l

MgSO₄ 0.5 g/l

CaCO₃ 5 g/l

Standard oligo elements 1 ml of each of the solutions A, B and C

Water 1000 ml

35 (pH adjusted to 6.9 after sterilization)

Solutions A, B and C as above.

The fermentation was performed at 28-30°C on a rotary shaker. After 24 hours the appropriate precursor was added. The culture was centrifuged after 72 hours and samples of 50 microliter of the broth were analyzed for the T-A2 major components concentration.

40 The analysis was performed according to the following HPLC method:

a. Separation by gradient reverse phase partition

Instrument: pump Varian 5000 A;

45 detector Varian at 254 nanometer;

injector: Rheodyne model 7125;

integrator: Spectra Physics model 4000;

Column: Zorbax[®] ODS 5 micrometer, 4.6 x 150 mm; (Du Pont)

Mobile Phase: A) CH₃CN: 0.025 M NaH₂PO₄ 1:9, pH 6.0

50 B) CH₃CN: 0.025 M NaH₂PO₄ 7:3, pH 6.0

Gradient profile: linear from 0% of B to 50% of B in 30 min. Flow rate 2 ml/min.

Injection: 50 microliter of fermentation broth

Retention times (minutes)

T-A2-1 = 16.9

55 T-A2-2 = 18.0

T-A2-3 = 18.6

T-A2-4 = 20.5

T-A2-5 = 20.9

Internal standard: 3,5-dihydroxytoluene (r.t. 6.3 minutes).

60

b. Percentage distribution

The components were separated by the above procedure and their relative distribution was obtained as a percent of the total of the five peaks by the area percentage method.

65

Additions g/l	Total yield microgram/l	T-A2-1 %	T-A2-2 %	T-A2-3 %	T-A2-4 %	T-A2-5 %
None	340	2.0	30.4	18.1	26.1	23.3
None	305	2.1	32.0	20.6	20.1	25.0
None	379	1.8	32.9	20.4	24.1	20.7
L-Valine (sodium salt)						
0.5	295	1.5	52.1	20.5	14.0	11.9
1	648	0.8	70.4	12.4	9.8	6.7
2	795	0.9	83.0	9.2	3.0	3.3
L-Isoleucine (sodium salt)						
0.5	116	2.0	29.3	20.4	32.1	16.2
1	134	1.8	24.1	13.5	40.2	20.4
2	159	1.7	13.5	4.4	66.5	13.9
L-Leucine (sodium salt)						
0.5	373	2.1	37.9	15.2	15.1	29.7
1	459	2.0	35.0	17.7	9.5	35.8
2	281	1.7	35.4	9.5	8.0	45.4
2.5	144	0.8	12.6	12.0	1.5	73.5

Additions g/l	Total yield microgram/l	T-A2-1 %	T-A2-2 %	T-A2-3 %	T-A2-4 %	T-A2-5 %
L-valine (HC1)						
2.5	750	0.2	86.2	8.7	2.8	2.1
Tri-oleine (containing 10% by-weight of tri-linoleine)						
2	320	12.0	17.2	41.0	18.9	10.9
4	258	12.3	16.1	48.8	16.9	10.9
Tri-linoleine						
2	341	29.0	20.3	14.0	20.1	17.1
5	355	31.2	22.9	17.4	16.9	11.5

By operating according to the above procedure in a further set of experiments the following data were obtained.

Additions g/l	Total yield microgram/l	T-A2-1 %	T-A2-2 %	T-A2-3 %	T-A2-4 %	T-A2-5 %
None	460	2.2	44.1	20.8	15.2	17.7
Isobutanol						
1	480	2.6	52.3	16.8	15.9	12.4
2	258	1.4	60.7	15.2	13.1	9.6
2-Methylbutanol						
1	423	2.3	42.1	15.2	26.3	14.1
2	265	1.7	46.3	10.5	30.1	11.4

Example 2

General Procedure

- 5 Actinoplanes teichomyceticus nov. sp. ATCC 31121 was pre-cultivated in a 500 ml shake flask containing 100 ml of the following medium:
- Meat extract 3 g/l
Tryptone 5 g/l
Yeast extract 5 g/l
10 Glucose 1 g/l
Soluble starch 24 g/l
Calcium carbonate 5 g/l
Water 1000 ml
(pH adjusted to 6.7 after sterilization)
- 15 The flasks were shaken for 24 hours at 28-30°C and then the pre-culture was used to inoculate jar fermentors each containing 10 liters of the following nutrient medium:
- Meat extract 4 g/l
Peptone 4 g/l
Yeast extract 1 g/l
20 Sodium chloride 2.5 g/l
Soybean meal 10 g/l
Glucose 50 g/l
Calcium carbonate 5 g/l
Tap water q.s. to 1000 ml
25 (pH adjusted to 6.9 after sterilization)
- The fermentors were incubated aerobically under stirring for 24 hours then the appropriate precursor was added. The fermentation was continued for further 90 hours, then the fermentors were harvested. Samples of broth (100 ml) were filtered at pH 11 (the pH was adjusted by the addition of 20% (w/v) sodium hydroxide) and analyzed according to the procedure described under Example 1 by injecting 40 microliter of each filtered sample solution whose pH was adjusted to 7.38 with a 0.1 M phosphate buffer.

Additions g/l	Total yield microgram/l	T-A2-1 %	T-A2-2 %	T-A2-3 %	T-A2-4 %	T-A2-5 %
None	678	4.5	50.9	16.0	15.8	12.8
None	684	3.5	50.0	15.1	17.0	14.4
Tri-linoleine						
5	764	15.5	45.1	14.0	12.8	12.7
10	596	52.8	21.1	10.4	5.5	10.2
Tri-oleine						
5	802	4.0	44.0	26.9	13.8	11.3
10	792	5.0	29.3	49.2	9.8	6.7
Methyl ester of 2-methylbutyric acid						
1	720	2.8	40.3	15.2	31.0	10.7
3	846	4.2	40.9	11.9	35.7	7.3
5	530	5.7	41.3	9.8	37.2	6.2

Additions g/l	Total yield microgram/l	T-A2-1 %	T-A2-2 %	T-A2-3 %	T-A2-4 %	T-A2-5 %
2-Methylbutyric acid (sodium salt)						
0.5	531	3.8	39.6	14.6	32.3	9.7
1.5	357	5.0	35.9	10.6	41.4	7.1
Methyl isobutyrate						
1	683	3.9	67.9	15.9	6.7	5.6
3	402	2.6	80.8	12.5	1.8	2.3
5	220	3.1	80.8	12.2	1.6	2.3
Isobutyric acid (sodium salt)						
0.5	532	3.8	69.1	18.4	4.4	4.3
1.5	214	3.3	79.7	13.5	1.8	1.7
L-Valine (buffered solution)						
1	458	2.3	79.3	10.2	4.3	3.9
2	377	3.2	83.9	9.4	1.6	1.9
3	250	1.9	85.3	9.6	1.2	2.0
Oil Additions						
Additions g/l	Total yield microgram/l	T-A2-1 %	T-A2-2 %	T-A2-3 %	T-A2-4 %	T-A2-5 %
Cotton seed oil						
10	571	37.3	26.4	22.9	6.0	7.4
Lard						
10	708	8.1	36.1	36.3	11.3	8.2
Soybean oil						
10	637	39.9	23.8	21.6	7.4	7.3
Sun flower oil						
10	712	31.7	31.8	18.6	8.8	9.1

For comparative purpose myristic acid, tripalmitin and tristearin were added to three jar fermentors at the concentration of 1 g/l, 5 g/l and 10 g/l respectively under the same conditions as above. No increasing effect of any of the T-A2 major components ratios was observed.

Example 3

Actinoplanes teichomyceticus nov. sp. ATCC 31121 was pre-cultivated as described in Example 2. The flasks of the preculture were used to inoculate a jar fermentor containing 10 liters of the nutrient medium reported in Example 2.

The fermentor was incubated aerobically under stirring at 25°C for 24 hours and then 2 g/l L-valine were added. The L-valine had previously been dissolved in water (2 g/15 ml) by adding sulfuric acid to reach pH 3 and the obtained solution had been stirred at 120°C for 10 minutes.

The fermentation was continued at 25°C for further 50 hours then the fermentor was harvested.

The broth filtered at pH 11 and analyzed according to the procedure described in Example 1, contained 220 microgram/l of T-A2 having the following composition:
T-A2-1: 2%; T-A2-2: 95%; T-A2-3: 3%.

Claims

1. A process for preparing teicoplanin A₂ (T-A2) selectively enriched in any of its major components T-A2-1, T-A2-2, T-A2-3, T-A2-4 or T-A2-5 which comprises adding to the culture medium of Actinoplanes teichomyceticus nov. sp. ATCC 31121, or one of its mutants which may produce T-A2 through the same

metabolic pathway, a selectively effective amount of an appropriate precursor of the respective acyl group of the glucosamine moiety of T-A2, as follows:

- a) the appropriate precursor for increasing the ratio of T-A2-1 in T-A2 complex is selected from linoleic acid, its salts with bases which are non-toxic to the microorganism and its esters with mono- and poly-hydroxy lower alkanols
 - b) the appropriate precursor for increasing the ratio of T-A2-2 in T-A2 complex is selected from valine, its salts with acids and bases which are non-toxic to the microorganism, alpha-keto-isovaleric acid, its salts with bases which are non-toxic to the microorganism, its esters with mono- and poly-hydroxy lower alkanols, isobutyric acid, its salts with bases which are non-toxic to the microorganism, its esters with mono- and poly-hydroxy lower alkanols, isobutanol and its esters with acids which are non-toxic to the microorganism
 - c) the appropriate precursor for increasing the ratio of T-A2-3 in T-A2 complex is selected from oleic acid, its salts with bases which are non-toxic to the microorganism, and its esters with mono- and poly-hydroxy lower alkanols
 - d) the appropriate precursor for increasing the ratio of T-A2-4 in T-A2 complex is selected from isoleucine, its salts with acids and bases which are non-toxic to the microorganism, alpha-keto-beta-methylvaleric acid, its salts with bases which are non-toxic to the microorganism, its esters with mono- and poly-hydroxy lower alkanols, 2-methylbutyric acid, its salts with bases which are non-toxic to the microorganism, its esters with mono- and poly-hydroxy lower alkanols, 2-methylbutanol and its esters with acids which are non-toxic to the microorganism
 - e) the appropriate precursor for increasing the ratio of T-A2-5 in T-A2 complex is selected from leucine, its salts with acids and bases which are non-toxic to the microorganism, isovaleric acid, its salts with bases which are non-toxic to the microorganism, its esters with mono- and poly-hydroxy lower alkanols, alpha-keto-isocaproic acid, its salts with bases which are non-toxic to the microorganism, its esters with mono- and poly-hydroxy lower alkanols, isoamyl alcohol and its esters with acids which are non-toxic to the microorganism.
2. A process as claimed in claim 1 wherein the appropriate precursor added is linoleic acid or its salts with bases non-toxic to the microorganism and the respective selectively effective amount ranges between 0.1 g/l and 2.5 g/l, preferably between 0.3 g/l and 1.5 g/l.
 3. A process as claimed in claim 1 wherein the appropriate precursor added is an ester of linoleic acid with a mono- or poly-hydroxy lower alkanol and the respective selectively effective amount ranges between 0.5 g/l and 15 g/l, preferably between 1 g/l and 5 g/l.
 4. A process as claimed in claim 1 wherein the appropriate precursor added is valine or its salts with acids and bases non-toxic to the microorganism and the respective selectively effective amount ranges between 0.5 g/l and 5 g/l, preferably between 1 g/l and 3 g/l.
 5. A process as claimed in claim 1 wherein the appropriate precursor added is isobutyric acid or its salts with bases non-toxic to the microorganism and the respective selectively effective amount ranges between 0.1 g/l and 2.5 g/l, preferably between 0.3 g/l and 1.5 g/l.
 6. A process as claimed in claim 1 wherein the appropriate precursor added is an ester of isobutyric acid with a mono- or poly-hydroxy lower alkanol and the respective selectively effective amount ranges between 0.5 g/l and 15 g/l, preferably between 1 g/l and 5 g/l.
 7. A process as claimed in claim 1 wherein the appropriate precursor added is isobutanol or its esters with acids non-toxic to the microorganism and the respective selectively effective amount ranges between 0.5 g/l and 5 g/l, preferably between 1 g/l and 2 g/l.
 8. A process as claimed in claim 1 wherein the appropriate precursor added is oleic acid or its salts with bases non-toxic to the microorganism and the respective selectively effective amount ranges between 0.1 g/l and 2.5 g/l, preferably between 0.3 g/l and 1.5 g/l.
 9. A process as in claim 1 wherein the appropriate precursor added is an ester of oleic acid with a mono- or poly-hydroxy lower alkanol and the respective selectively effective amount ranges between 0.5 g/l and 15 g/l, preferably between 1 g/l and 5 g/l.
 10. A process as claimed in claim 1 wherein the appropriate precursor added is isoleucine or its salts with acids and bases non-toxic to the microorganism and the respective selectively effective amount ranges between 0.5 g/l and 5 g/l, preferably between 1 g/l and 3 g/l.
 11. A process as claimed in claim 1 wherein the appropriate precursor added is 2-methylbutyric acid or its salts with bases non-toxic to the microorganism and the respective selectively effective amount ranges between 0.1 g/l and 2.5 g/l, preferably between 0.3 g/l and 1.5 g/l.
 12. A process as claimed in claim 1 wherein the appropriate precursor added is an ester of 2-methylbutyric acid with a mono- or poly-hydroxy lower alkanol and the respective selectively effective amount ranges between 0.5 g/l and 15 g/l, preferably between 1 g/l and 5 g/l.
 13. A process as claimed in claim 1 wherein the appropriate precursor added is 2-methylbutanol or its ester with an acid non-toxic to the microorganism and the respective selectively effective amount ranges between 0.5 g/l and 5 g/l, preferably between 1 g/l and 2 g/l.
 14. A process as claimed in claim 1 wherein the appropriate precursor added is leucine or its salts with acids and bases non-toxic to the microorganism and the respective selectively effective amount ranges between 0.5 g/l and 5 g/l, preferably between 1 g/l and 3 g/l.

15. A process as claimed in claim 1 wherein the appropriate precursor added is isovaleric acid or its salts with bases non-toxic to the microorganism and the respective selectively effective amount ranges between 0.1 g/l and 2.5 g/l, preferably between 0.3 g/l and 1.5 g/l.

16. A process as claimed in claim 1 wherein the appropriate precursor added is an ester of isovaleric acid with a mono- or poly-hydroxy lower alkanol and the respective selectively effective amount ranges between 0.5 g/l and 15 g/l, preferably between 1 g/l and 5 g/l.

17. A process as claimed in claim 1 wherein the appropriate precursor added is isoamyl alcohol or its esters with acids non-toxic to the microorganism and the respective selectively effective amount ranges between 0.5 g/l and 5 g/l, preferably between 1 g/l and 2 g/l.

18. A process as claimed in claim 1 wherein the appropriate precursor added is alpha-keto-isovaleric acid, its salts with bases non-toxic to the microorganism or its esters with mono- or poly-hydroxy lower alkanols and the respective selectively effective amount ranges between 0.5 g/l and 5 g/l, preferably between 1 g/l and 3 g/l.

19. A process as claimed in claim 1 wherein the appropriate precursor added is alpha-keto-beta-methylvaleric acid, its salts with bases non-toxic to the microorganism or its esters with mono- or poly-hydroxy lower alkanols and the respective selectively effective amount ranges between 0.5 g/l and 5 g/l, preferably between 1 g/l and 3 g/l.

20. A process as claimed in claim 1 wherein the appropriate precursor added is alpha-keto-isocaproic acid, its salts with bases non-toxic to the microorganism, or its esters with mono- or poly-hydroxy lower alkanols and the respective selectively effective amount ranges between 0.5 g/l and 5 g/l, preferably between 1 g/l and 3 g/l.

21. A process as claimed in any one of the claims 2, 4, 5, 8, 10, 11, 14, 15, 18, 19 and 20 where the salts with bases non-toxic to the microorganism are sodium or ammonium salts.

22. A process as claimed in any one of the claims 3, 6, 9, 12, 16, 18, 19 and 20 wherein the ester is an ester with one of the following alkanols: methanol, ethanol, propanol, ethylene glycol and glycerol.

23. A process as claimed in any one of the claims 4, 10 and 14 wherein the amino acid is in the L- form.

24. A process as claimed in any one of the claims 4, 10, 14 and 23 wherein the salt with an acid non-toxic to the microorganism is the hydrochloride or the sulphate.

25. A process as claimed in any one of the claims 7, 13 and 17 wherein the ester with an acid non-toxic to the microorganism is an ester with one of the following acids: acetic acid, propionic acid and butyric acid.

26. A process as claimed in any one of the claims 1, 2, 3, 8 and 9 wherein the unsaturated fatty acids or their esters are added as natural raw materials containing said acids or their glycerides.

27. A process as claimed in any one of the preceding claims wherein the strain is Actinoplanes teichomyceticus nov. sp. ATCC 31121.

28. A process as claimed in any one of the preceding claims wherein the fermentation is carried out at a temperature between 25°C and 35°C, and preferably between 27°C and 35°C.

29. A process as claimed in any one of the preceding claims wherein the addition of the appropriate precursor is carried out 24 to 48 hours after the fermentation is started.

30. In a process for enriching teicoplanin A₂ in any of its major components, i.e. T-A2-1, T-A2-2, T-A2-3, T-A2-4, and/or T-A2-5, the improvement which consists of adding to the culture medium of Actinoplanes teichomyceticus nov. sp. ATCC 31121, or one of its mutants which may produce T-A2 through the same metabolic pathway, a selectively effective amount of an appropriate precursor of the respective acyl group of the glucosamine moiety of T-A2, is as follows:

a) the appropriate precursor for increasing the ratio of T-A2-1 in T-A2 complex is selected from linoleic acid, its salts with bases which are non-toxic to the microorganism and its esters with mono- and poly-hydroxy lower alkanols

b) the appropriate precursor for increasing the ratio of T-A2-2 in T-A2 complex is selected from valine, its salts with acids and bases which are non-toxic to the microorganism, alpha-keto-isovaleric acid, its salts with bases which are non-toxic to the microorganism, its esters with mono- and poly-hydroxy lower alkanols, isobutyric acid, its salts with bases which are non-toxic to the microorganism, its esters with mono- and poly-hydroxy lower alkanols, isobutanol and its esters with acids which are non-toxic to the microorganism

c) the appropriate precursor for increasing the ratio of T-A2-3 in T-A2 complex is selected from oleic acid, its salts with bases which are non-toxic to the microorganism, its esters with mono- and poly-hydroxy lower alkanols

d) the appropriate precursor for increasing the ratio of T-A2-4 in T-A2 complex is selected from isoleucine, its salts with acids and bases which are non-toxic to the microorganism, alpha-keto-beta-methylvaleric acid, its salts with bases which are non-toxic to the microorganism, its esters with mono- and poly-hydroxy lower alkanols, 2-methylbutyric acid, its salts with bases which are non-toxic to the microorganism, its esters with mono- and poly-hydroxy lower alkanols, 2-methylbutanol and its esters with acids which are non-toxic to the microorganism

e) the appropriate precursor for increasing the ratio of T-A2-5 in T-A2 complex is selected from leucine, its salts with acids and bases which are non-toxic to the microorganism, isovaleric acid, its salts with bases which are non-toxic to the microorganism, its esters with mono- and poly-hydroxy lower al-

kan Is, alpha-keto-isocaproic acid, its salts with bases which are non-toxic to the microorganism, its esters with mono- and poly-hydroxy lower alkanols, isoamyl alcohol and its esters with acids which are non-toxic to the microorganism.

31. A process according to claim 1 for enriching teicoplanin A₂ in its components 2, 4 or 5 in a percentage up to 95% which comprises adding a selectively effective amount of valine, isoleucine, or leucine, respectively, to the fermentation medium.

Patentansprüche

1. Verfahren zur Herstellung von Teicoplanin A₂ (T-A₂), das mit einer seiner Hauptkomponenten T-A₂-1, T-A₂-2, T-A₂-3, T-A₂-4 oder T-A₂-5 selektiv angereichert ist, umfassend die Zugabe einer selektiv wirksamen Menge einer geeigneten Vorstufe des entsprechenden Arylrestes des Glucosamin-Telles von T-A₂ zum Kulturmedium von *Actinoplanes teichomyceticus* nov. sp. ATCC 31121 oder einer seiner Mutanten, die T-A₂ über denselben Stoffwechselweg produzieren können, wobei wie folgt:
 - a) die geeignete Vorstufe zur Erhöhung des Antelles von T-A₂-1 im T-A₂-Komplex Linolsäure, eines ihrer Salze mit Basen, die für den Mikroorganismus ungiftig sind, oder einer ihrer Ester mit Mono- und Polyhydroxy-Niederalkanolen ist,
 - b) die geeignete Vorstufe zur Erhöhung des Antelles von T-A₂-2 im T-A₂-Komplex Valin, eines seiner Salze mit Säuren und Basen, die für den Mikroorganismus ungiftig sind, α -Ketoisovaleriansäure, eines ihrer Salze mit Basen, die für den Mikroorganismus ungiftig sind, einer ihrer Ester mit Mono- und Polyhydroxy-Niederalkanolen, Isobuttersäure, eines ihrer Salze mit Basen, die für den Mikroorganismus ungiftig sind, einer ihrer Ester mit Mono- und Polyhydroxy-Niederalkanolen, Isobutanol oder einer seiner Ester mit Säuren, die für den Mikroorganismus ungiftig sind, ist,
 - c) die geeignete Vorstufe zur Erhöhung des Antelles von T-A₂-3 im T-A₂-Komplex Ölsäure, eines ihrer Salze mit Basen, die für den Mikroorganismus ungiftig sind, oder einer ihrer Ester mit Mono- und Polyhydroxy-Niederalkanolen ist,
 - d) die geeignete Vorstufe zur Erhöhung des Antelles von T-A₂-4 im T-A₂-Komplex Isoleucin, eines seiner Salze mit Säuren und Basen, die für den Mikroorganismus ungiftig sind, α -Keto- β -methylvaleriansäure, eines ihrer Salze mit Basen, die für den Mikroorganismus ungiftig sind, einer ihrer Ester mit Mono- und Polyhydroxy-Niederalkanolen, 2-Methylbuttersäure, eines ihrer Salze mit Basen, die für den Mikroorganismus ungiftig sind, einer ihrer Ester mit Mono- und Polyhydroxy-Niederalkanolen, 2-Methylbutanol oder einer seiner Ester mit Säuren, die für den Mikroorganismus ungiftig sind, ist,
 - e) die geeignete Vorstufe zur Erhöhung des Antelles von T-A₂-5 im T-A₂-Komplex Leucin, eines seiner Salze mit Säuren und Basen, die für den Mikroorganismus ungiftig sind, Isovaleriansäure, eines ihrer Salze mit Basen, die für den Mikroorganismus ungiftig sind, einer ihrer Ester mit Mono- und Polyhydroxy-Niederalkanolen, α -Ketoisocaproinsäure, eines ihrer Salze mit Basen, die für den Mikroorganismus ungiftig sind, einer ihrer Ester mit Mono- und Polyhydroxy-Niederalkanolen, Isoamylalkohol oder einer seiner Ester mit Säuren, die für den Mikroorganismus ungiftig sind, ist.
2. Verfahren nach Anspruch 1, wobei die geeignete Vorstufe, die zugegeben wird, aus Linolsäure oder ihren Salzen mit für den Mikroorganismus ungiftigen Basen besteht und die entsprechende selektiv wirksame Menge im Bereich 0,1 g/l bis 2,5 g/l, vorzugsweise von 0,3 g/l bis 1,5 g/l, liegt.
3. Verfahren nach Anspruch 1, wobei die geeignete Vorstufe, die zugegeben wird, ein Ester der Linolsäure mit einem Mono- oder Polyhydroxy-Niederalkanol ist und die entsprechende selektiv wirksame Menge im Bereich von 0,5 g/l bis 15 g/l, vorzugsweise von 1 g/l bis 5 g/l, liegt.
4. Verfahren nach Anspruch 1, wobei die geeignete Vorstufe, die zugegeben wird, aus Valin oder seinen Salzen mit Säuren und Basen, die für den Mikroorganismus ungiftig sind, besteht und die entsprechende selektiv wirksame Menge im Bereich von 0,5 g/l und 5 g/l, vorzugsweise von 1 g/l und 3 g/l, liegt.
5. Verfahren nach Anspruch 1, wobei die geeignete Vorstufe, die zugegeben wird, aus Isobuttersäure oder ihren Salzen mit für den Mikroorganismus ungiftigen Basen besteht und die entsprechende selektiv wirksame Menge im Bereich von 0,1 g/l bis 2,5 g/l, vorzugsweise von 0,3 g/l bis 1,5 g/l, liegt.
6. Verfahren nach Anspruch 1, wobei die geeignete Vorstufe, die zugegeben wird, ein Ester von Isobuttersäure mit einem Mono- oder Polyhydroxy-Niederalkanol ist und die entsprechende selektiv wirksame Menge im Bereich von 0,5 g/l bis 15 g/l, vorzugsweise von 1 g/l bis 5 g/l, liegt.
7. Verfahren nach Anspruch 1, wobei die geeignete Vorstufe, die zugegeben wird, aus Isobutanol oder seinen Estern mit für den Mikroorganismus ungiftigen Säuren besteht und die entsprechende selektiv wirksame Menge im Bereich von 0,5 g/l bis 5 g/l, vorzugsweise von 1 g/l bis 2 g/l, liegt.
8. Verfahren nach Anspruch 1, wobei die geeignete Vorstufe, die zugegeben wird, aus Ölsäure oder ihren Salzen mit für den Mikroorganismus ungiftigen Basen besteht und die entsprechende selektiv wirksame Menge im Bereich von 0,1 g/l bis 2,5 g/l, vorzugsweise von 0,3 g/l bis 1,5 g/l, liegt.
9. Verfahren nach Anspruch 1, wobei die geeignete Vorstufe, die zugegeben wird, ein Ester von Ölsäure mit einem Mono- oder Polyhydroxy-Niederalkanol ist und die entsprechende selektiv wirksame Menge im Bereich von 0,5 g/l bis 15 g/l, vorzugsweise von 1 g/l bis 5 g/l, liegt.
10. Verfahren nach Anspruch 1, wobei die geeignete Vorstufe, die zugegeben wird, aus Isoleucin oder seinen Salzen mit Säuren oder Basen, die für den Mikroorganismus ungiftig sind, besteht und die ent-

sprechend selektiv wirksame Menge im Bereich von 0,5 g/l bis 5 g/l, vorzugsweise von 1 g/l bis 3 g/l, liegt.

11. Verfahren nach Anspruch 1, wobei die geeignete Vorstufe, die zugegeben wird, aus 2-Methylbuttersäure oder ihren Salzen mit für den Mikroorganismus ungiftigen Basen besteht und die entsprechende selektiv wirksame Menge im Bereich von 0,1 g/l bis 2,5 g/l, vorzugsweise von 0,3 g/l bis 1,5 g/l, liegt.

12. Verfahren nach Anspruch 1, wobei die geeignete Vorstufe, die zugegeben wird, ein Ester von 2-Methylbuttersäure mit einem Mono- oder Polyhydroxy-Niederalkanol ist und die entsprechende selektiv wirksame Menge im Bereich von 0,5 g/l bis 15 g/l, vorzugsweise von 1 g/l bis 5 g/l, liegt.

13. Verfahren nach Anspruch 1, wobei die geeignete Vorstufe, die zugegeben wird, 2-Methylbutanol oder sein Ester mit einer für den Mikroorganismus ungiftigen Säure ist und die entsprechende selektiv wirksame Menge im Bereich von 0,5 g/l bis 5 g/l, vorzugsweise von 1 g/l bis 2 g/l, liegt.

14. Verfahren nach Anspruch 1, wobei die geeignete Vorstufe, die zugegeben wird, aus Leucin oder seinen Salzen mit Säuren oder Basen, die für den Mikroorganismus ungiftig sind, besteht und die entsprechende selektiv wirksame Menge im Bereich von 0,5 g/l bis 5 g/l, vorzugsweise von 1 g/l bis 3 g/l, liegt.

15. Verfahren nach Anspruch 1, wobei die geeignete Vorstufe, die zugegeben wird, aus Isovaleriansäure oder ihren Salzen mit für den Mikroorganismus ungiftigen Basen besteht und die entsprechende selektiv wirksame Menge im Bereich von 0,1 g/l bis 2,5 g/l, vorzugsweise von 0,3 g/l bis 1,5 g/l, liegt.

16. Verfahren nach Anspruch 1, wobei die geeignete Vorstufe, die zugegeben wird, ein Ester von Isovaleriansäure mit einem Mono- oder Polyhydroxy-Niederalkanol ist und die entsprechende selektiv wirksame Menge im Bereich von 0,5 g/l bis 15 g/l, vorzugsweise von 1 g/l bis 5 g/l, liegt.

17. Verfahren nach Anspruch 1, wobei die geeignete Vorstufe, die zugegeben wird, aus Isoamylalkohol oder seinem Ester mit für den Mikroorganismus ungiftigen Säuren besteht und die entsprechende selektiv wirksame Menge im Bereich von 0,5 g/l bis 5 g/l, vorzugsweise von 1 g/l bis 2 g/l, liegt.

18. Verfahren nach Anspruch 1, wobei die geeignete Vorstufe, die zugegeben wird, aus α -Ketoisovaleriansäure, ihren Salzen mit für den Mikroorganismus ungiftigen Basen oder ihren Estern mit Mono- oder Polyhydroxy-Niederalkanolen besteht und die entsprechende selektiv wirksame Menge im Bereich von 0,5 g/l bis 5 g/l, vorzugsweise von 1 g/l bis 3 g/l, liegt.

19. Verfahren nach Anspruch 1, wobei die geeignete Vorstufe, die zugegeben wird, aus α -Keto- β -methylvaleriansäure, ihren Salzen mit für den Mikroorganismus ungiftigen Basen oder ihren Estern mit Mono- oder Polyhydroxy-Niederalkanolen besteht und die entsprechende selektiv wirksame Menge im Bereich von 0,5 g/l bis 5 g/l, vorzugsweise von 1 g/l bis 3 g/l, liegt.

20. Verfahren nach Anspruch 1, wobei die geeignete Vorstufe, die zugegeben wird, aus α -Ketolocaproinsäure, ihren Salzen mit für den Mikroorganismus ungiftigen Basen oder ihren Estern mit Mono- oder Polyhydroxy-Niederalkanolen besteht und die entsprechende selektiv wirksame Menge im Bereich von 0,5 g/l bis 5 g/l, vorzugsweise von 1 g/l bis 3 g/l, liegt.

21. Verfahren nach einem der Ansprüche 2, 4, 5, 8, 10, 11, 14, 15, 18, 19 und 20, wobei die Salze mit für den Mikroorganismus ungiftigen Basen Natrium- oder Ammoniumsalze sind.

22. Verfahren nach einem der Ansprüche 3, 6, 9, 12, 16, 18, 19 und 20, wobei der Ester ein Ester mit einem der folgenden Alkanole, nämlich Methanol, Äthanol, Propanol, Äthylenglycol und Glycerin, ist.

23. Verfahren nach einem der Ansprüche 4, 10 und 14, wobei die Aminosäure in der L-Form vorliegt.

24. Verfahren nach einem der Ansprüche 4, 10, 14 und 23, wobei das Salz mit einer für den Mikroorganismus ungiftigen Säure das Hydrochlorid oder Sulfat ist.

25. Verfahren nach einem der Ansprüche 7, 13 und 17, wobei der Ester mit einer für den Mikroorganismus ungiftigen Säure ein Ester mit einer der folgenden Säuren, nämlich Essigsäure, Propionsäure und Buttersäure, ist.

26. Verfahren nach einem der Ansprüche 1, 2, 3, 8 und 9, wobei die ungesättigten Fettsäuren oder ihre Ester als natürliche Rohstoffe, welche diese Säuren oder ihre Glyceride enthalten, zugegeben werden.

27. Verfahren nach einem der vorstehenden Ansprüche, wobei der Stamm *Actinoplanes telchomyceticus* nov. sp. ATCC 31121 ist.

28. Verfahren nach einem der vorstehenden Ansprüche, wobei die Fermentation bei einer Temperatur zwischen 25°C und 35°C, vorzugsweise zwischen 27°C und 35°C, durchgeführt wird.

29. Verfahren nach einem der vorstehenden Ansprüche, wobei die geeignete Vorstufe 24 bis 48 Stunden nach Beginn der Fermentation zugegeben wird.

30. In einem Verfahren zur Anreicherung von *Tetraplanin A₂* mit einer seiner Hauptkomponenten, d.h. T-A2-1, T-A2-2, T-A2-3, T-A2-4 und/oder T-A2-5, besteht die Verbesserung in der Zugabe einer selektiv wirksamen Menge einer geeigneten Vorstufe des entsprechenden Acylrestes des Glucosamin-Teiles von T-A2 zum KulturmEDIUM von *Actinoplanes telchomyceticus* nov. sp. ATCC 31121 oder einer seiner Mutanten, die T-A2 über denselben Stoffwechselweg produzieren können, wobei wie folgt

a) die geeignete Vorstufe zur Erhöhung des Anteiles von T-A2-1 im T-A2-Komplex Linolsäure, eines ihrer Salze mit Basen, die für den Mikroorganismus ungiftig sind, oder einer ihrer Ester mit Mono- und Polyhydroxy-Niederalkanolen ist,

b) die geeignete Vorstufe zur Erhöhung des Anteils von T-A2-2 im T-A2-Komplex Valin, eines seiner Salze mit Säuren und Basen, die für den Mikroorganismus ungiftig sind, α -Ketoisovaleriansäure, el-

nes ihrer Salze mit Basen, die für den Mikroorganismus ungiftig sind, einer ihrer Ester mit Mono- und Polyhydroxy-Niederalkanolen, Isobuttersäure, eines ihrer Salze mit Basen, die für den Mikroorganismus ungiftig sind, einer ihrer Ester mit Mono- und Polyhydroxy-Niederalkanolen, Isobutanol oder eines seiner Ester mit Säuren, die für den Mikroorganismus ungiftig sind, ist

- 5 c) die geeignete Vorstufe zur Erhöhung des Antelles von T-A2-3 im T-A2-Komplex Ölsäure, eines ihrer Salze mit Basen, die für den Mikroorganismus ungiftig sind, einer ihrer Ester mit Mono- und Polyhydroxy-Niederalkanolen ist,
- 10 d) die geeignete Vorstufe zur Erhöhung des Antelles von T-A2-4 mit T-A2-Komplex Isoleucin, eines seiner Salze mit Säuren und Basen, die für den Mikroorganismus ungiftig sind, α -Keto- β -methylvaleriansäure, eines ihrer Salze mit Basen, die für den Mikroorganismus ungiftig sind, einer ihrer Ester mit Mono- und Polyhydroxy-Niederalkanolen, 2-Methylbuttersäure, eines ihrer Salze mit Basen, die für den Mikroorganismus ungiftig sind, einer ihrer Ester mit Mono- und Polyhydroxy-Niederalkanolen, 2-Methylbutanol oder einer seiner Ester mit Säuren, die für den Mikroorganismus ungiftig sind, ist
- 15 e) die geeignete Vorstufe zur Erhöhung des Antelles von T-A2-5 im T-A2-Komplex Leucin, eines seiner Salze mit Säuren und Basen, die für den Mikroorganismus ungiftig sind, Isovaleriansäure, eines ihrer Salze mit Basen, die für den Mikroorganismus ungiftig sind, einer ihrer Ester mit Mono- und Polyhydroxy-Niederalkanolen, α -Ketolsocaprionsäure, eines ihrer Salze mit Basen, die für den Mikroorganismus ungiftig sind, einer ihrer Ester mit Mono- und Polyhydroxy-Niederalkanolen, Isoamylalkohol
- 20 oder einer seiner Ester mit Säuren, die für den Mikroorganismus ungiftig sind, ist.

31. Verfahren nach Anspruch 1 zur Anreicherung von Teicoplanin A₂ mit seinen Komponenten 2, 4 oder 5 in einem Prozentsatz von bis zu 95%, das die Zugabe einer selektiv wirksamen Menge von Valin, Isoleucin oder Leucin zum Fermentationsmedium umfaßt.

25 Revendications

1. Procédé pour préparer une téicoplanine A₂ (T-A2) enrichie sélectivement en l'un quelconque de ses constituants principaux T-A2-1, T-A2-2, T-A2-3, T-A2-4 ou T-A2-5, qui comprend l'addition au milieu de culture d'Actinoplanes telchomyceticus nov. sp. ATCC 31121, ou de l'un de ses mutants qui peut produire

30 T-A2 par la même voie métabolique, d'une quantité sélectivement efficace d'un précurseur approprié du groupe acyle respectif de la partie glucosamine de T-A2, de la manière suivante:

- a) le précurseur approprié pour augmenter le rapport de T-A2-1 dans le complexe T-A2 est choisi parmi l'acide linoléique, ses sels avec des bases qui sont non toxiques pour le micro-organisme et ses esters avec des alcanols inférieurs mono- et polyhydroxylés
- 35 b) le précurseur approprié pour augmenter le rapport de T-A2-2 dans le complexe T-A2 est choisi parmi la valine, ses sels avec des acides et des bases qui sont toxiques pour le micro-organisme, l'acide α -cétoisovalérique, ses sels avec des bases qui sont non toxiques pour le micro-organisme, ses esters avec des alcanols inférieurs mono- et polyhydroxylés, l'acide isobutyrique, ses sels avec des bases qui sont non toxiques pour le micro-organisme, ses esters avec des alcanols inférieurs mono- et polyhydroxylés, l'isobutanol et ses esters avec des acides qui sont non toxiques pour le micro-organisme
- 40 c) le précurseur approprié pour augmenter le rapport de T-A2-3 dans le complexe T-A2 est choisi parmi l'acide oléique, ses sels avec des bases qui sont non toxiques pour le micro-organisme, ses esters avec des alcanols inférieurs mono- et polyhydroxylés
- 45 d) le précurseur approprié pour augmenter le rapport de T-A2-4 dans le complexe T-A2 est choisi parmi l'isoleucine, ses sels avec des acides et des bases qui sont non toxiques pour le micro-organisme, l'acide α -céto- β -methylvalérique, ses sels avec des bases qui sont non toxiques pour le micro-organisme, ses esters avec des alcanols inférieurs mono- et polyhydroxylés, l'acide 2-méthylbutyrique, ses sels avec des bases qui sont non toxiques pour le micro-organisme, ses esters avec des alcanols inférieurs mono- et polyhydroxylés, le 2-méthylbutanol et ses esters avec des acides qui sont non toxiques pour le micro-organisme
- 50 e) le précurseur approprié pour augmenter le rapport de T-A2-5 dans le complexe T-A2 est choisi parmi la leucine, ses sels avec des acides et des bases qui sont non toxiques pour le micro-organisme, l'acide isovalérique, ses sels avec des bases qui sont non toxiques pour le micro-organisme, ses esters avec des alcanols inférieurs mono- et polyhydroxylés, l'acide α -céto-isocaproïque, ses sels avec des bases qui sont non toxiques pour le micro-organisme, ses esters avec des alcanols inférieurs mono- et polyhydroxylés, l'alcool isoamylique et ses esters avec des acides qui sont non toxiques pour le micro-organisme.
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2. Procédé selon la revendication 1, dans lequel le précurseur approprié ajouté est l'acide linoléique ou ses sels avec des bases non toxiques pour le micro-organisme et la quantité sélectivement efficace respective est comprise entre 0,1 g/l et 2,5 g/l, de préférence entre 0,3 g/l et 1,5 g/l.

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3. Procédé selon la revendication 1, dans lequel le précurseur approprié ajouté est un ester de l'acide linoléique avec un alcanol inférieur mono- ou polyhydroxylé et la quantité sélectivement efficace respective est comprise entre 0,5 g/l et 15 g/l, de préférence entre 1 g/l et 5 g/l.

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4. Procédé selon la revendication 1, dans lequel le précurseur approprié ajouté est la valine ou ses

- sels avec des acides et des bases non toxiques pour le micro-organisme et la quantité sélectivement efficace respective est comprise entre 0,5 g/l et 5 g/l, de préférence entre 1 g/l et 3 g/l.
- 5 Procédé selon la revendication 1, dans lequel le précurseur approprié ajouté est l'acide isobutyrique ou ses sels avec des bases non toxiques pour le micro-organisme et la quantité sélectivement efficace respective est comprise entre 0,1 g/l et 2,5 g/l, de préférence entre 0,3 g/l et 1,5 g/l.
- 6 Procédé selon la revendication 1, dans lequel le précurseur approprié ajouté est un ester de l'acide isobutyrique avec un alcool inférieur mono- ou polyhydroxylé et la quantité sélectivement efficace respective est comprise entre 0,5 g/l et 15 g/l, de préférence entre 1 g/l et 5 g/l.
- 10 7. Procédé selon la revendication 1, dans lequel le précurseur approprié ajouté est l'isobutanol ou ses esters avec des acides non toxiques pour le micro-organisme et la quantité sélectivement efficace respective est comprise entre 0,5 g/l et 5 g/l, de préférence entre 1 g/l et 2 g/l.
8. Procédé selon la revendication 1, dans lequel le précurseur approprié ajouté est l'acide oléique ou ses sels avec des bases non toxiques pour le micro-organisme et la quantité sélectivement efficace respective est comprise entre 0,1 g/l et 2,5 g/l, de préférence entre 0,3 g/l et 1,5 g/l.
- 15 9. Procédé selon la revendication 1, dans lequel le précurseur approprié ajouté est un ester de l'acide oléique avec un alcool inférieur mono- ou polyhydroxylé et la quantité sélectivement efficace respective est comprise entre 0,5 g/l et 15 g/l, de préférence entre 1 g/l et 5 g/l.
10. Procédé selon la revendication 1, dans lequel le précurseur approprié ajouté est l'isoleucine ou ses sels avec des acides et des bases non toxiques pour le micro-organisme et la quantité sélectivement efficace respective est comprise entre 0,5 g/l et 5 g/l, de préférence entre 1 g/l et 3 g/l.
- 20 11. Procédé selon la revendication 1, dans lequel le précurseur approprié ajouté est l'acide 2-méthylbutyrique ou ses sels avec des bases non toxiques pour le micro-organisme et la quantité sélectivement efficace respective est comprise entre 0,1 g/l et 2,5 g/l, de préférence entre 0,3 g/l et 1,5 g/l.
12. Procédé selon la revendication 1, dans lequel le précurseur approprié ajouté est un ester de l'acide 2-méthylbutyrique avec un alcool inférieur mono- ou polyhydroxylé et la quantité sélectivement efficace respective est comprise entre 0,5 g/l et 15 g/l, de préférence entre 1 g/l et 5 g/l.
- 25 13. Procédé selon la revendication 1, dans lequel le précurseur approprié ajouté est le 2-méthylbutanol ou son ester avec un acide non toxique pour le micro-organisme et la quantité sélectivement efficace respective est comprise entre 0,5 g/l et 5 g/l, de préférence entre 1 g/l et 2 g/l.
- 30 14. Procédé selon la revendication 1, dans lequel le précurseur approprié ajouté est la leucine ou ses sels avec des acides et des bases non toxiques pour le micro-organisme et la quantité sélectivement efficace respective est comprise entre 0,5 g/l et 5 g/l, de préférence entre 1 g/l et 3 g/l.
15. Procédé selon la revendication 1, dans lequel le précurseur approprié ajouté est l'acide isovalérique ou ses sels avec des bases non toxiques pour le micro-organisme et la quantité sélectivement efficace respective est comprise entre 0,1 g/l et 2,5 g/l, de préférence entre 0,3 g/l et 1,5 g/l.
- 35 16. Procédé selon la revendication 1, dans lequel le précurseur approprié ajouté est l'acide isovalérique ou ses esters avec des alcools inférieurs mono- ou polyhydroxylés et la quantité sélectivement efficace respective est comprise entre 0,5 g/l et 15 g/l, de préférence entre 1 g/l et 5 g/l.
17. Procédé selon la revendication 1, dans lequel le précurseur approprié ajouté est l'alcool isoamylique ou ses esters avec des acides non toxiques pour le micro-organisme et la quantité sélectivement efficace respective est comprise entre 0,5 g/l et 5 g/l, de préférence entre 1 g/l et 2 g/l.
- 40 18. Procédé selon la revendication 1, dans lequel le précurseur approprié ajouté est l'acide alpha-cétoisovalérique, ses sels avec des bases non toxiques pour le micro-organisme ou ses esters avec des alcools inférieurs mono- ou polyhydroxylés, et la quantité sélectivement efficace respective est comprise entre 0,5 g/l et 5 g/l, de préférence entre 1 g/l et 3 g/l.
- 45 19. Procédé selon la revendication 1, dans lequel le précurseur approprié ajouté est l'acide alpha-céto-bêta-méthylvalérique, ses sels avec des bases non toxiques pour le micro-organisme ou ses esters avec des alcools inférieurs mono- ou polyhydroxylés, et la quantité sélectivement efficace respective est comprise entre 0,5 g/l et 5 g/l, de préférence entre 1 g/l et 3 g/l.
- 50 20. Procédé selon la revendication 1, dans lequel le précurseur approprié ajouté est l'acide alpha-céto-isocaproïque, ses sels avec des bases non toxiques pour le micro-organisme ou ses esters avec des alcools inférieurs mono- ou polyhydroxylés, et la quantité sélectivement efficace respective est comprise entre 0,5 g/l et 5 g/l, de préférence entre 1 g/l et 3 g/l.
21. Procédé selon l'une quelconque des revendications 2, 4, 5, 8, 10, 11, 14, 15, 18, 19 et 20, dans lequel les sels avec des bases non toxiques pour le micro-organisme sont des sels de sodium ou d'ammonium.
- 55 22. Procédé selon l'une quelconque des revendications 3, 6, 9, 12, 16, 18, 19 et 20, dans lequel l'ester est un ester avec l'un des alcools suivants: méthanol, éthanol, propanol, éthylène glycol et glycérol.
23. Procédé selon l'une quelconque des revendications 4, 10 et 14, dans lequel l'acide est sous la forme L.
- 60 24. Procédé selon l'une quelconque des revendications 4, 10, 14 et 23, dans lequel le sel avec un acide non toxique pour le micro-organisme est le chlorhydrate ou le sulfate.
25. Procédé selon l'une quelconque des revendications 7, 13, et 17, dans lequel l'ester avec un acide non toxique pour le micro-organisme est un ester avec l'un des acides suivants: acide acétique, acide propionique et acide butyrique.
- 65 26. Procédé selon l'une quelconque des revendications 1, 2, 3, 8 et 9, dans lequel les acides gras in-

saturés ou leurs esters sont ajoutés sous forme de matières premières naturelles contenant ces acides ou leurs glycérides.

27. Procédé selon l'une quelconque des revendications précédentes, dans lequel la souche est *Actinoplanes teichomyceticus* nov. sp. ATCC 31121.

28. Procédé selon l'une quelconque des revendications précédentes, dans lequel la fermentation est effectuée à une température comprise entre 25 et 35°C, et de préférence entre 27°C et 35°C.

29. Procédé selon l'une quelconque des revendications précédentes, dans lequel l'addition du précurseur approprié est effectuée 24 à 48 heures après le démarrage de la fermentation.

30. Dans un procédé pour enrichir la téicoplanine A₂ en l'un quelconque de ses constituants principaux, à savoir T-A2-1, T-A2-2, T-A2-3, T-A2-4, et/ou T-A2-5, le perfectionnement qui consiste à ajouter au milieu de culture d'*Actinoplanes teichomyceticus* nov. sp. ATCC 31121, ou d'un de ses mutants qui peut produire T-2 par la même voie métabolique, une quantité sélectivement efficace d'un précurseur approprié du groupe acyle respectif de la partie glucosamine de T-A2, de la manière suivante:

a) le précurseur approprié pour augmenter le rapport de T-A2-1 dans le complexe T-A2 est choisi parmi l'acide linoléique, ses sels avec des bases qui sont non toxiques pour le micro-organisme et ses esters avec des alcanols inférieurs mono- et polyhydroxylés

b) le précurseur approprié pour augmenter le rapport de T-A2-2 dans le complexe T-A2 est choisi parmi la valine, ses sels avec des acides et des bases qui sont non toxiques pour le micro-organisme, l'acide alpha-céto-isovalérique, ses sels avec des bases qui sont non toxiques pour le micro-organisme, ses esters avec des alcanols inférieurs mono- et polyhydroxylés, l'acide isobutyrique, ses sels avec des bases qui sont non toxiques pour le micro-organisme, ses esters avec des alcanols inférieurs mono- et polyhydroxylés, l'isobutanol et ses esters avec des acides qui sont non toxiques pour le micro-organisme

c) le précurseur approprié pour augmenter le rapport T-A2-3 dans le complexe T-A2 est choisi parmi l'acide oléique, ses sels avec des bases qui sont non toxiques pour le micro-organisme, ses esters avec des alcanols inférieurs mono- et polyhydroxylés

d) le précurseur approprié pour augmenter le rapport de T-A2-4 dans le complexe T-A2 est choisi parmi l'isoleucine, ses sels avec des acides et des bases qui sont non toxiques pour le micro-organisme, l'acide alpha-céto-bêta-méthylvalérique, ses sels avec des bases qui sont non toxiques pour le micro-organisme, ses esters avec des alcanols inférieurs mono- et polyhydroxylés, l'acide 2-méthylbutyrique, ses sels avec des bases qui sont non toxiques pour le micro-organisme, ses esters avec des alcanols inférieurs mono- et polyhydroxylés, le 2-méthylbutanol et ses esters avec des acides qui sont non toxiques pour le micro-organisme

e) le précurseur approprié pour augmenter le rapport de T-A2-5 dans le complexe T-A2 est choisi parmi la leucine, ses sels avec des acides et des bases qui sont non toxiques pour le micro-organisme, l'acide isovalérique, ses sels avec des bases qui sont non toxiques pour le micro-organisme, ses esters avec des alcanols inférieurs mono- et polyhydroxylés, l'acide alpha-céto-isocaproïque, ses sels avec des bases qui sont non toxiques pour le micro-organisme, ses esters avec des alcanols inférieurs mono- et polyhydroxylés, l'alcool isoamylique et ses esters avec des acides qui sont non toxiques pour le micro-organisme.

31. Procédé selon la revendication 1, pour enrichir la téicoplanine A₂ en ses constituants 2, 4 ou 5 dans un pourcentage allant jusqu'à 95%, qui comprend l'addition d'une quantité sélectivement efficace de valine, d'isoleucine ou de leucine, respectivement, au milieu de fermentation.